REACTIVITIES OF GIBBERELLINS WITH A CATIONIC EXCHANGE RESIN, DOWEX-50W

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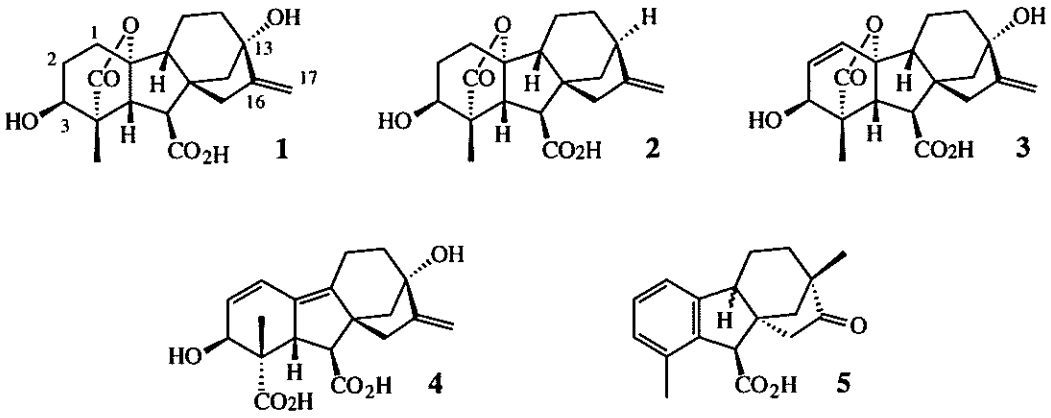
Abstract—A cationic exchange resin containing sulfonic acid functional groups, Dowex-50W, controlled the reactions of gibberellins (GAs) catalyzed by acid: by optimizing the reaction temperature and solvent, GA_1 (**1**) furnished gibberellin C (**7**) quantitatively, while GA_4 (**2**) gave *endo*-GA_4 (**10**) or 16-*O*-methylGA_2 (**12**) in moderate to good yields, respectively; in a refluxing methanol, GA_3 methyl ester (**14**) gave triene carboxylic acid (**15**) (47%), *iso*-GA_3 methyl ester (**16**) (22%) and diene carboxylic acid (**17**) (11%). The formation of **15** and **16** was explained by a reaction mechanism *via* a common intermediate, 2β,3β-epoxide (**18**).

INTRODUCTION

In previous studies on the structure identification of natural gibberellins (GAs) and their synthesis, the susceptible nature of GAs to acidic conditions has been well documented, which comes from their high functionalities and strained carbon skeleton.[1] The exocyclic $\Delta^{16(17)}$-double bond of 13-hydroxyGAs, represented by GA_1 (**1**) and GA_3 (**3**), undergoes a Wagner-Meerwein rearrangement with inversion of the C/D ring configuration, *e.g.*, **1** to gibberellin C (**7**) in Scheme 1,[2] while that of 13-deoxyGAs represented by GA_4 (**2**) undergoes hydration and double bond migration to the endocyclic 15(16)-position, *i.e.*, **2** to GA_2 (**11**) and *endo*-GA_4 (**10**) in Scheme 2.[3] On the other hand, the $\Delta^{1(2)}$-double bond on **3** is much more susceptible. Even in unbuffered aqueous solution at room temperature, **3** is autocatalytically transformed to gibberellenic acid (**4**), although the rate is very slow,[4] and under more vigorous or acidic conditions, **3** affords a variety of products including gibberic acid (**5**) through a complex degradation cascade.[5] Since controlling these reactions is so difficult, under most acidic conditions, we always face the formation of an awkward complex mixture of rearrangement and degradation products along with the starting material and target compound.

In our early work,[6] we found that Dowex-50W resin efficiently removed the methoxymethyl (MOM) protecting group of 3- and 13-hydroxyl functions on 3,13-dihydroxyGAs in refluxing aqueous methanol without rearrangement of the C/D ring system. In contrast, the rearrangement was observed to a

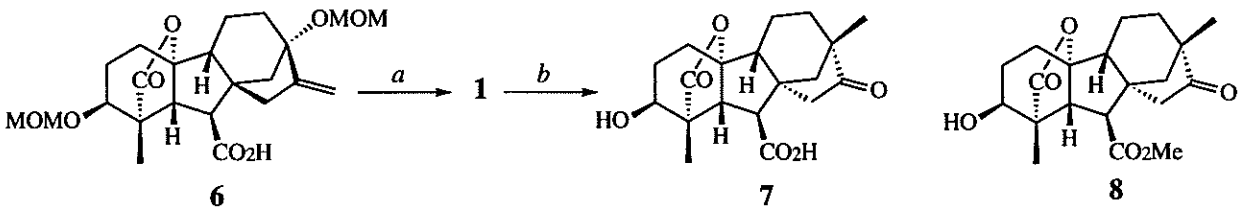


considerable extent when *p*-toluenesulfonic acid was used. This suggested that Dowex-50W, a cationic exchange resin containing sulfonic acid functional groups attached to a styrene-divinylbenzene copolymer, was mild compared with the corresponding monomeric congener. With a view to elucidating the scope and limitation for controlling the reactions of GAs under acidic conditions, we examined the reactivities of typical natural GAs, **1** and **2**, and GA_3 methyl ester (**14**) with Dowex-50W resin.

RESULTS AND DISCUSSION

As reported earlier,[6] on treatment of 3,13-di-*O*-MOM-GA_1 (**6**)[7] with Dowex-50W resin in a mixed solution of methanol (MeOH) and water (H_2O), 5:1, at 70 °C for 6 h, hydrolytic removal of the MOM groups occurred smoothly to give GA_1 (**1**) in 91% yield, where the formation of gibberellin C (**7**) was not detected at all. Elongation of the reaction time to 16 h did not affect this reaction, pointing to the completely stable nature of the C/D ring system on 13-hydroxyGAs under these conditions. However, elevating the reaction temperature gradually caused the Wagner-Meerwein rearrangement of the C/D ring system: when **1** was treated with the resin in MeOH and H_2O, 2:5, at 105 °C for 7 h, the rearrangement was completed to give **7** (84%) along with a considerable amount of its methyl ester (**8**) (8.6%). As the better conditions for preparation of **7**, GA_1 (**1**) was treated with the resin in dioxane-methanol-H_2O (2:1:2) at 105 °C for 22 h, affording **7** in 96% yield.

Scheme 1



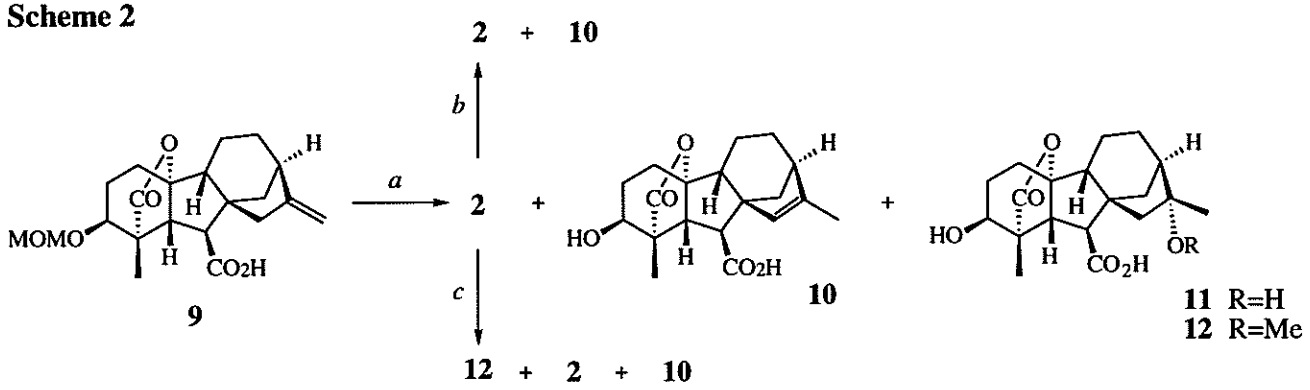
a: Resin, MeOH-H_2O (5:1), 70 °C, 6 h. *b:* Resin, dioxane-MeOH-H_2O (2:1:2), 105 °C, 22 h.

In contrast, the application of Dowex-50W resin for selective removal of the MOM group on 3-*O*-MOM-GA_4 (**9**)[7] [Dowex resin, MeOH-H_2O, 5:1, 70 °C, 10 h], unexpectedly failed, where the double bond migration to the endocyclic 15(16)-position and addition of the protic solvent to the exocyclic $\Delta^{16(17)}$-double bond occurred to give a mixture of GA_4 (**2**) and its double bond isomer, *endo*-GA_4 (**10**), along

with hydrated GA_4, or GA_2 (**11**), and its 16-*O*-methyl ether (**12**). This evidenced that the *exo*-methylene of 13-deoxyGAs was more susceptible than that of 13-hydroxyGAs to acid. The low reactivity of *exo*-methylene of 13-hydroxyGAs may be ascribed to the induction effect of the 13-hydroxyl function which decreases the nucleophilicity of the double bond. Since compounds (**10**) and (**12**) are of interest with respect to their biological activity, optimization of the reaction conditions for the preparation of each compound was attempted.

On treatment of **2** with the resin in refluxing tetrahydrofuran (THF) for 15 h, a 3:7 mixture of **2** and **10** was obtained in 92% yield. Elongation of the reaction time to 24 h had no effect on the product ratio, and under the same conditions, **10** also gave the same mixture, indicating that the ratio was that in equilibrium. On the other hand, when the reaction was carried out in refluxing MeOH, **2** afforded **12** in 50% yields and a 3:7 mixture of **2** and **10** in 39% yield. Elongation of the reaction time was not effective for increasing the formation of **12**, suggesting that addition and elimination of methanol were reversible under the conditions and the reaction was stationary at this stage.

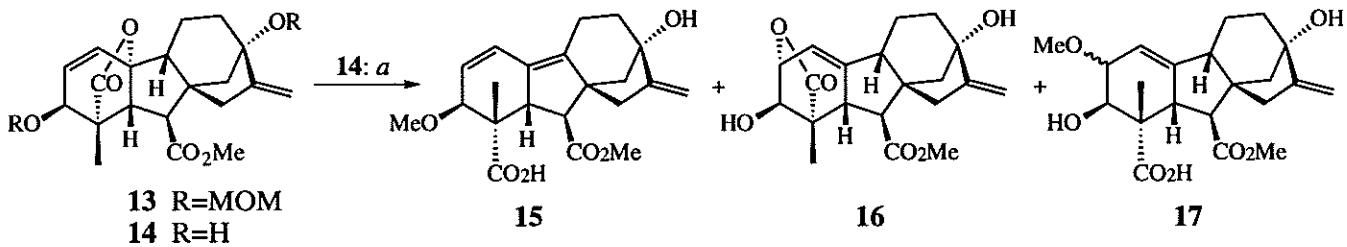
Scheme 2



a: Resin, MeOH-H_2O (5:1), 70 °C, 10 h. *b:* Resin, THF, reflux, 15 h. *c:* Resin, MeOH, reflux, 6 h.

Application of the Dowex resin for selective removal of the MOM groups on 3,13-di-*O*-MOM-GA_3 methyl ester (**13**)[7] [Dowex resin, MeOH-H_2O, 5:1, reflux, 6 h] also failed, giving a product mixture speculated to mainly consist of triene carboxylic acid (**15**), *iso*-GA_3 methyl ester (**16**) and diene carboxylic acid (**17**) along with a considerable amount of unidentified products. To simplify the reaction, GA_3 methyl ester (**14**) was treated with the resin in MeOH at refluxing temperature for 4 h, giving **15**, **16** and **17** in 47, 22 and 11% yields, respectively, where **17** was obtained as a single compound but the stereochemistry at the C-2 remains unclear (*vide infra*). These compounds were not interchangeable under the same condition.

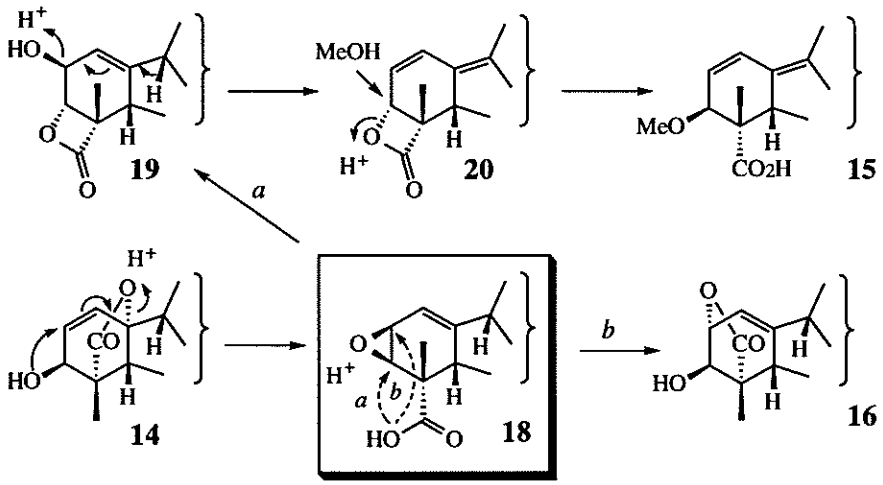
Scheme 3



a: Resin, MeOH, reflux, 4 h.

The formation of compounds (**15**) and (**16**) may be plausibly explained by a reaction mechanism *via* a common intermediate, 2β,3β-epoxide (**18**), as illustrated in Scheme 4. The C(10)-O(10a) bond fission of **14**, facilitated by acid, initiates the intramolecular *anti*-S_N2' displacement of the acyloxyl group of the bridged lactone by the 3-hydroxyl group, leading to the formation of **18**. The subsequent intramolecular nucleophilic attack of the carboxyl function to the oxirane ring, catalyzed by acid, takes place in two competitive ways leading to reaction paths *a* and *b*. In path *a*, the carboxyl function attacks the C-3 position to give β-lactone (**19**), which is then converted to **20** by dehydration. The terminating step of path *a* is the S_N2 attack of methanol to the C-3, furnishing the end product (**15**). On the other hand, in path *b*, the attack of the carboxyl function at the C-2 furnishes **16**.

Scheme 4



This is the first description of the reaction mechanism of acidic degradation of GA_3 congeners by postulating 2β,3β-epoxide like **18** as an intermediate to final products,[8] where the observed selectivity in cyclization of **18** is consistent with the general tendency,[9] *i.e.*, 4-*exo* cyclization (**18**→**19**) predominated over 5-*endo*/7-*exo* one (**18**→**16**). In this connection, it is well documented that the intermediary carboxylate of 2β,3β-epoxide (**18**) is implicated in the transformation of **14** to **16** under weakly alkali condition (0.01 M aqueous KOH), where the alkoxide of **14** initiates the intramolecular *anti*-S_N2' reaction.[10] However, the selectivity in the subsequent cyclization of the resulting carboxylate anion of **18** is entirely different from that under acidic conditions: 5-*endo*/7-*exo* cyclization takes place exclusively to give **16** only.

The structures of new compounds (**12**, **15** and **17**) were verified by MS and NMR spectroscopy. NMR experiments on these compounds and **16** were carried out with 600 MHz by PFG-DQFCOSY, PFG-HMQC and PFG-HMBC, by which all resonances were completely assigned (see EXPERIMENTAL). The α-configuration of 16-OMe on **12** was attested to by the 1D selective PFG-ROESY experiment: methyl protons of 16-OMe [δ 3.15 (s)] showed ROE with 15α-H [δ 1.82 (d, J_{gem}=13.7 Hz)], while 17-H_3 [δ 1.33 (s)] with 15β-H [δ 1.57 (dd, J_{gem}=13.7 Hz and $J_{15\beta,14\alpha}$=2.4 Hz)]. The β-configuration of 3-OMe on **15** was deduced from the NOE differential experiment: no NOE effect was observed between 3-H [δ 3.91 (d, $J_{3,2}$=5.4 Hz)] and 5-H [δ 3.59 (dd, $J_{5,6}$=8.8 and $J_{5,11\beta}$=4.4 Hz)] with β-configuration. Also, no NOE effect between 3-H [δ 4.07 (d, $J_{3,2}$=4.4 Hz)] and 5-H [δ 3.05 (br d, $J_{5,6}$=6.8 Hz)] indicated the β-

configuration of 3-OH on **17**. However, the stereochemistry at C-2 of **17** could not be defined by NMR analysis. The 2-H resonanced at δ 3.80 with coupling constants, $J_{2,3}$=4.4, $J_{2,1}$=3.4, $J_{2,9}$=2.5 and $J_{2,5}$=1.5 Hz, and NOEs were observed between 3-H and both of 2-H and methyl protons of the 2-OMe [δ 3.46 (s)]. These data did not rule out possibilities of α- and β-configurations of 2-OMe on **17** possessing A-ring with a half-chair conformation.

CONCLUSION

As described, the application of Dowex-50W resin for removal of the MOM protecting group of 3- and/or 13-hydroxyl function on GAs was found to be limited to GA_1 congeners only. However, the moderate acidity of the resin allowed us to confirm that the susceptibilities of acid-sensitive moieties typical of natural GAs were in the following order: $\Delta^{1(2)}$-double bond of GA_3 congeners > $\Delta^{16(17)}$-double bond of 13-deoxyGAs > $\Delta^{16(17)}$-double bond of 13-hydroxyGAs. In a refluxing methanol solution, the resin effectively controlled the reaction of a GA_3 congener: **14** gave a mixture of **15**-**17** in a good combined yield without further degradation. This substantiated the implication of 2β,3β-epoxide intermediate like **18** in acidic degradation of GA_3 congeners. By varying the reaction temperature and/or solvent, gibberellin C (**7**) from **1** and *endo*-GA_4 (**10**) or 16-*O*-methylGA_2 (**12**) from **2** were obtained in moderate to good yields, respectively. Since these compounds are of interest with respect to the structure-activity relationships of GAs, the evaluation of a variety of GA activities of these compounds is being undertaken.

EXPERIMENTAL

General.—Melting points were determined on Yanagimoto micromelting point apparatus and are uncorrected. NMR measurements were performed on JEOL JNM-A600 or Bruker AC-300 spectrometer. All spectra were recorded using standard pulse sequences. Chemical shifts were recorded as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0 ppm) for ^{1}H and the solvent (δ 77.0 ppm) for ^{13}C as an internal reference in $CDCl_3$ solution; or relative to the solvent: δ 2.06 ppm for ^{1}H and 29.80 ppm for ^{13}C, in acetone-d_6 solution. All *J*-values are given in Hz. MS spectra, EI-MS and FAB-MS, were obtained with JEOL-SX102 and JEOL-HX-110 mass spectrometers. Analytical TLC was conducted on micro-slides coated with Merck Kieselgel KG60F-254; the developed plates were stained with 10% (w/v) vanillin in concentrated sulfuric acid at 180 °C. All reactions were carried out under a nitrogen atmosphere. Column chromatography was conducted using Wakogel C-300 [Wako Pure Chemical Industries] as the adsorbent. The ratios of mixed solvents were v/v.

General procedure for reaction of GAs with Dowex resin.—Purchased resin, Dowex-50W-X2 (H^+ form), was used after successively washed with dist. H_2O, 2N NaOH, dist. H_2O and 2N HCl, then dist. H_2O on a glass-filter until the filtrate was neutral (wet resin); especially for anhydrous reactions, the resin was further washed with MeOH and Et_2O, and was dried *in vacuo* (dry resin). Resin was added to a solution of a substrate and the heterogeneous mixture was stirred with heating until the starting material disappeared or the reaction reached a stationary state, monitored by TLC. The resin was filtered off through a glass-filter and washed with methanol. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel.

Removal of MOM protecting groups of 3,13-di-O-MOM-GA$_1$ (**6**).—3,13-di-*O*-MOM-GA$_1$ (**6**)[7] (100 mg, 0.23 mmol) in MeOH (2.5 mL) and water (0.5 mL) was treated with wet resin (200 mg) at 70 °C for 6 h. Column chromatography using AcOEt as the eluent afforded GA$_1$ (**1**) (73 mg, 91%), whose R_f value and ^{1}H and ^{13}C NMR spectra[11] were identical to those of authentic sample gifted by Prof. T. Sassa.

Reaction of GA$_1$ (**1**) [*Preparation of Gibberellin C* (**7**)].—GA$_1$ (**1**) (49 mg, 0.14 mmol) in a mixture of dioxane (1 mL), MeOH (0.5 mL) and H_2O (1 mL) was treated with wet resin (50 mg) at 105 °C for 22 h. Column chromatography using AcOEt-AcOH (50:1) as the eluent gave gibberellin C (**7**) (47 mg, 96%) as colorless prisms: mp 241-244 °C (decomp) (AcOEt) [lit.,[2] mp 265-267 °C (decomp)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (3H, s, 18-H$_3$), 1.27 (3H, s, 17-H$_3$), 2.14 (1H, d, J=18.8 Hz, 14β-H), 2.68 (1H, d, J=6.7 Hz, 6-H), 3.01 (1H, dd, J=18.8 and 3.9 Hz, 14α-H), 3.22 (1H, d, J=6.7 Hz, 5-H), 3.91 (1H, m, 3-H); ^{13}C NMR (75 MHz, acetone-d_6) δ 14.88 and 19.93 (C-17 and -18), 20.00, 26.71, 28.86, 36.19, 48.26 and 50.93 (C-1, -2, -11. -12, -14 and -15), 51.21, 53.28 and 54.23 (C-5, -6 and -9), 49.35, 51.37 and 54.34 (C-4, -8 and -16), 70.92 (C-3), 92.96 (C-10), 174.54 and 178.56 (C-7 and -19), 217.99 (C-13); EI-MS *m/z* 348 (M$^+$, 74%), 330 (M$^+$-H_2O, 49), 302 (100).
On the other hand, GA$_1$ (**1**) (39 mg, 0.11 mmol) in MeOH (2 mL) and water (5 mL) was treated with wet resin (1.0 g) at refluxing temperature for 7 h. Column chromatography using AcOEt as the eluent afforded gibberellin C methyl ester (**8**) (3.5 mg, 8.6%) as colorless prisms: mp 215-218 °C (AcOEt-hexane) [lit.,[2] mp 226-228 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (3H, s, 18-H$_3$), 1.23 (3H, s, 17-H$_3$), 2.11 (1H, d, J=18.9 Hz, 14β-H), 2.64 (1H, d, J=6.7 Hz, 6-H), 3.00 (1H, dd, J=18.9 and 3.9 Hz, 14α-H), 3.22 (1H, d, J=6.7 Hz, 5-H), 3.74 (3H, s, -CO_2CH_3), 3.89 (1H, m, 3-H); EI-MS *m/z* 362 (M$^+$, 94%), 344 (M$^+$-H_2O, 88), 316 (100). Then, elution with AcOEt-AcOH (50:1) gave **7** (33 mg, 84%). The methyl ester of **7**, prepared by esterification with ethereal diazomethane in methanol at 0 °C, gave the same R_f value and ^{1}H NMR spectra as those of **8**.

Reaction of 3-O-MOM-GA$_4$ (**9**).—3-*O*-MOM-GA$_4$ (**9**)[7] (20 mg, 0.053 mmol) in MeOH (0.5 mL) and H_2O (0.1 mL) was treated with wet resin (50 mg) at 70 °C for 10 h. Column chromatography using AcOEt–CH_2Cl_2 (5:1) as the eluent afforded a 3:7 mixture of GA$_4$ (**2**) and *endo*-GA$_4$ (**10**) (4.8 mg, 24%), and elution with AcOEt gave 16α-*O*-methylGA$_2$ (**12**) (5.3 mg, 27%). The further elution with AcOEt-AcOH (100:1) gave GA$_2$ (**11**) (3.4 mg, 18%) as a colorless amorphous powder [lit.,[3] mp 256-258 °C (decomp)]: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (3H, s, 18-H$_3$), 1.38 (3H, s, 17-H$_3$), 2.68 (1H, d, J=10.8 Hz, 6-H), 3.03 (1H, d, J=10.8 Hz, 5-H), 3.82 (1H, br s, 3-H); EI-MS *m/z* 350 (M$^+$, 2%), 332 (M$^+$-H_2O, 57), 270 (100). The R_f value and ^{1}H NMR spectra of **11** were identical to those of authentic sample prepared by the method described by Aldridge *et al.*[3]

Reaction of GA$_4$ (**2**).—(a) *Preparation of* endo-*GA$_4$* (**10**): GA$_4$ (**2**) (100 mg, 0.30 mmol) in dry THF (5 mL) was treated with dry resin (100 mg) at refluxing temperature for 15 h. The crude mixture was shown to consist of **2** and *endo*-GA$_4$ (**10**) in a ratio of 29:71 by the ^{1}H NMR spectrum. Column chromatography using AcOEt-CH_2Cl_2 (2:1) as the eluent afforded *endo*-GA$_4$ (**10**) (56 mg, 56%) as colorless prisms: mp 228-232 °C (decomp) (AcOEt-MeOH-hexane) [lit.,[3] mp 256-260 °C (decomp)]; ^{1}H NMR (300 MHz, acetone-d_6 with a few drops of D_2O) δ 1.09 (3H, s, 18-H$_3$), 1.26 (1H, ddd, J=13.6, 12.4 and 7.1 Hz), 2.14 (1H, d, J=10.1 Hz), 2.20 (1H, dd, J=7.8 and 4.7 Hz), 2.42 (1H, d, J=9.9 Hz, 6-H), 3.10 (1H, d, J=9.9 Hz, 5-H), 3.68 (1H, br d, J=2.8 Hz, 3-H), 5.46 (1H, br s, 15-H); ^{13}C NMR (75 MHz, acetone-d_6) δ 15.12 and 15.33 (C-17 and -18), 18.93, 19.98, 27.11, 29.02 and 41.70 (C-1, -2, -11. -12 and -14), 41.89, 48.00, 52.22 and 52.77 (C-5, -6, -9 and -13), 55.55 and 56.20 (C-4 and -8), 70.27 (C-3), 94.15 (C-10), 133.51 (C-15), 149.30 (C-16), 174.19 and 178.78 (C-7 and -19); EI-MS *m/z* 332 (M$^+$, 100%), 270 (88). The further elution gave a mixture of **2** and **10** (36 mg, 36%).

(b) *Preparation of 16-O-methyl GA$_2$* (**12**): GA$_4$ (**2**) (93 mg, 0.28 mmol) in dry MeOH (5 mL) was treated with dry resin (100 mg) at refluxing temperature for 6 h. Column chromatography using AcOEt as the eluent afforded a 3: 7 mixture of **2** and *endo*-GA$_4$ (**10**) (37 mg, 39%), and then elution with AcOEt-AcOH (100:1) gave 16-*O*-methyl GA$_2$ (**12**) (51 mg, 50%) as colorless needles: mp 219-221 °C (decomp) (AcOEt); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.17 (3H, s, 18-H$_3$), 1.33 (3H, s, 17-H$_3$), 1.49 and 1.81 (each 1H, each m, 12-H$_2$), 1.57 (1H, dd, J=13.7 and 2.4 Hz, 15β-H), 1.57 and 1.73 (each 1H, each m, 11-H$_2$), 1.72 (1H, br d, J=11.2 Hz, 14α-H), 1.75 and 1.98 (each 1H, each m, 1-H$_2$), 1.81 (1H, m, 9-H), 1.82 (1H, d, J=13.7 Hz, 15α-H), 1.82 (2H, m, 2-H$_2$), 2.01 (1H, dd, J=11.2 and 4.9 Hz, 14β-H), 2.19 (1H, dd, J=8.8 and 4.9 Hz, 13-H), 2.69 (1H, d, J=10.7 Hz, 6-H), 3.08 (1H, d, J=10.7 Hz, 5-H), 3.15 (3H, s, 16-OCH_3), 3.83 (1H, dd, J=2.9 and 2.4 Hz, 3-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.66 (C-18), 16.02 (C-11), 21.40 (C-17), 22.06 (C-12), 27.22 (C-1), 27.92 (C-2), 35.91 (C-14), 39.35 (C-13), 49.68 (16-OCH_3), 50.83 (C-5), 51.60 (C-8), 52.16 (C-6), 52.54 (C-15), 54.56 (C-4), 54.64 (C-9), 70.28 (C-3), 86.86 (C-16), 94.15 (C-10), 175.20 (C-7), 178.27 (C-19); EI-MS *m/z* 364 (M$^+$, 37%), 332 (M$^+$-MeOH, 48), 270 (100); HR-EI-MS *m/z* [M]$^+$: Found, 364.1893. Calcd for $C_{20}H_{28}O_6$, 364.1886.

Reaction with GA$_3$ methyl ester (**14**).—GA$_3$ methyl ester (**14**) (99 mg, 0.28 mmol) in dry MeOH (2.5 mL) was treated with dry resin (100 mg) at 70 °C for 4 h. Column chromatography using CH_2Cl_2-AcOEt (10:1) as the eluent afforded *iso*-GA$_3$ methyl ester (**16**) (21 mg, 22%), and elution with CH_2Cl_2-AcOEt (2:1) afforded triene carboxylic acid (**15**) (49 mg, 47%). The further elution with AcOEt-AcOH (100:1) gave diene carboxylic acid (**17**) (12 mg, 11%).

Triene carboxylic acid (**15**): colorless prisms, mp 220-222 °C (decomp) (AcOEt); ^{1}H NMR (600 MHz, acetone-d_6) δ 1.20 (3H, s, 18-H$_3$), 1.67 (1H, m, 12β-H), 1.68 (1H, m, 14α-H), 1.72 (1H, m, 12α-H), 2.03 (1H, m, 11β-H), 2.13 (1H, dd, J=10.3 and 2.4 Hz, 14β-H), 2.16 (1H, br d, J=16.6 Hz, 15β-H), 2.24 (1H, ddd, J=16.6, 2.9 and 2.9 Hz, 15α-H), 2.61 (1H, dd, J=16.1 and 6.3 Hz, 11α-H), 3.39 (3H, s, 3-OCH_3), 3.59 (1H, dd, J=8.8 and 4.4 Hz, 5-H), 3.69 (3H, s, -CO_2CH_3), 3.74 (1H, d, J=8.8 Hz, 6-H), 3.91 (1H, d, J=5.4 Hz, 3-H), 4.90 (1H, br s, 17-H *syn to* C-15), 5.12 (1H, br s, 17-H *syn to* C-13), 6.06 (1H, dd, J=9.8 and 5.4 Hz, 2-H), 6.40 (1H, d, J=9.8 Hz, 1-H); ^{13}C NMR (150 MHz, acetone-d_6) δ 20.31 (C-18), 21.15 (C-11), 40.01 (C-15), 40.68 (C-12), 49.27 (C-5), 49.68 (C-4), 49.82 (C-6), 51.72 (-$CO_2\underline{C}H_3$), 52.98 (C-14), 56.72 (C-8), 57.31 (3-OCH_3), 78.63 (C-3), 79.02 (C-13), 105.85 (C-17), 125.62 (C-1), 127.98 (C-2), 128.08 (C-10), 140.45 (C-9), 155.98 (C-16), 175.43 (C-7), 175.78 (C-19); EI-MS *m/z* 374 (M$^+$, 14%), 297 (100); HR-EI-MS *m/z* [M]$^+$: Found, 374.1734. Calcd for $C_{21}H_{26}O_6$, 374.1729.

iso-GA$_3$ methyl ester (**16**)**:** a colorless amorphous powder [lit.,[2] mp 172-173 °C]; ^{1}H NMR (600 MHz, $CDCl_3$ with a few drops of D_2O) δ 1.20 (3H, s, 18-H$_3$), 1.39 (1H, dd, J=11.2 and 2.0 Hz, 14β-H), 1.55 (1H, dd, J=11.2 and 2.9 Hz, 14α-H), 1.56 and 1.76 (each 1H, each m, 12-H$_2$), 1.76 and 1.95 (each 1H, each m, 11-H$_2$), 2.24 (1H, br d, J=16.7 Hz, 15β-H), 2.57 (1H, d, J=5.9 Hz, 6-H), 2.59 (1H, ddd, J=16.7, 2.9 and 2.9 Hz, 15α-H), 2.66 (1H, br d, J=5.9 Hz, 9-H), 3.26 (1H, dd, J=5.9 and 2.9 Hz, 5-H), 3.74 (3H, s, -CO_2CH_3), 4.25 (1H, d, J=5.4 Hz, 3-H), 4.75 (1H, dd, J=5.4 and 5.4 Hz, 2-H), 4.98 (1H, dd, J=2.9 and 2.0 Hz, 17-H *syn* to C-15), 5.13 (1H, dd, J=2.9 and 2.0 Hz, 17-H *syn to* C-13), 5.79 (1H, ddd, J=5.4, 2.9 and 1.5 Hz, 1-H); ^{13}C NMR (150 MHz, $CDCl_3$ with a few drops of D_2O) δ 16.82 (C-18), 18.62 (C-11), 36.88 (C-12), 38.68 (C-15), 45.37 (C-9), 45.59 (C-5), 48.17 (C-4), 48.54 (C-14), 48.81 (C-6), 49.37 (C-8), 52.04 (-$CO_2\underline{C}H_3$), 73.63 (C-2), 75.17 (C-3), 78.76 (C-13), 106.86 (C-17), 113.52 (C-1), 152.13 (C-10), 153.34 (C-16), 175.09 (C-7), 176.69 (C-19); EI-MS *m/z* 360 (M$^+$, 8%), 397 (100).

Diene carboxylic acid (**17**): a colorless amorphous powder; ^{1}H NMR (600 MHz, $CDCl_3$ with a few drops of D_2O) δ 1.36 (3H, s, 18-H$_3$), 1.51 (1H, br dd, J=10.7 and 2.0 Hz, 14β-H), 1.57 and 1.78 (each 1H,

each m, 12-H_2), 1.72 and 1.94 (each 1H, each m, 11-H_2), 1.92 (1H, br d, J=10.7 Hz, 14α-H), 2.21 (1H, br d, J=16.1 Hz, 15β-H), 2.52 (1H, m, 9-H), 2.55 (1H, ddd, J=16.1, 2.9 and 2.9 Hz, 15α-H), 2.88 (1H, d, J=6.8 Hz, 6-H), 3.05 (1H, br d, J=6.8 Hz, 5-H), 3.46 (3H, s, 2-OCH_3), 3.71 (3H, s, -CO_2CH_3), 3.80 (1H, dddd, J=4.4, 3.4, 2.5 and 1.5 Hz, 2-H), 4.07 (1H, d, J=4.4 Hz, 3-H), 4.97 (1H, br s, 17-H *syn* to C-15), 5.12 (1H, dd, J=2.9 and 2.0 Hz, 17-H *syn to* C-13), 5.42 (1H, ddd, J=3.4, 2.5 and 2.0 Hz, 1-H); ^{13}C NMR (150 MHz, $CDCl_3$ with a few drops of D_2O) δ 21.11 (C-18), 18.77 (C-11), 37.49 (C-12), 39.33 (C-15), 46.21 (C-9), 46.33 (C-4), 47.28 (C-5), 48.80 (C-14), 49.76 (C-8), 50.26 (C-6), 51.78 (-$CO_2\underline{C}H_3$), 57.13 (2-OCH_3), 71.74 (C-3), 78.42 (C-2), 78.93 (C-13), 106.53 (C-17), 111.34 (C-1), 146.16 (C-10), 154.26 (C-16), 175.79 (C-7), 176.40 (C-19); EI-MS *m/z* 374 (M^+-H_2O, 7%), 297 (100); HR-FAB-MS *m/z* ([M+1]$^+$: positive ion, glycerol): Found, 393.1909. Calcd for $C_{21}H_{29}O_7$, 393.1913.

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